On page 1, below the title, please add the following:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. Application Serial No. 09/254,780, filed March 10, 1999, which is a national stage application under 35 U.S.C. § 371 of International Application No. PCT/US97/16187, filed September 12, 1997, which claims the benefit of U.S. Provisional Application Serial No. 60/054,528, filed August 4, 1997, and U.S. Provisional Application Serial No. 60/025,985, filed September 12, 1996, all of which are herein incorporated by reference in their entireties.

On page 17, please amend the first paragraph to read as follows:

Plants differ from microorganisms in that their messages do not possess a defined ribosome binding site. Rather, it is believed that ribosomes attach to the 5' end of the message and scan for the first available ATG at which to start translation. Nevertheless, it is believed that there is a preference for certain nucleotides adjacent to the ATG and that expression of microbial genes can be enhanced by the inclusion of a eukaryotic consensus translation initiator at the ATG. Clontech (1993/1994 catalog, page 210) have suggested the sequence GTCGACCATGGTC (SEQ ID NO:1) as a consensus translation initiator for the expression of the *E. coli uidA* gene in plants. Further, Joshi (NAR 15: 6643-6653 (1987)) has compared many plant sequences adjacent to the ATG and suggests the consensus TAAACAATGGCT (SEQ ID NO:2). In situations where difficulties are encountered in the expression of microbial ORFs in plants, inclusion of one of these sequences at the initiating ATG may improve translation. In such cases the last three nucleotides of the consensus may not be appropriate for inclusion in the modified sequence due to their modification of the second AA residue. Preferred sequences

adjacent to the initiating methionine may differ between different plant species. A survey of 14 maize genes located in the GenBank database provided the following results:

Position Before the Initiating ATG in 14 Maize Genes:

	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1
C	3	8	4	6	2	5	6	0	10	7
T	3	0	3	4	3	2	1	1	1	0
A	2	3	1	4	3	2	3	7	2	3
G	6	3	6	0	6	5	4	6	1	5

On page 26, please amend paragraphs 2 and 3 to read as follows:

pCGN1761ENX is cleaved with *SphI*, treated with T4 DNA polymerase and religated, thus destroying the *SphI* site located 5' to the double 35S promoter. This generates vector pCGN1761ENX/Sph-. pCGN1761ENX/Sph- is cleaved with *EcoRI*, and ligated to an annealed molecular adaptor of the sequence 5'-AATTCTAAAGCATGCCGATCGG-3' (SEQ ID NO:3) / 5'-AATTCCGATCGGCATGCTTTA-3' (SEQ ID NO:4). This generates the vector pCGNSENX which incorporates the *quasi*-optimized plant translational initiation sequence TAAA-C adjacent to the ATG which is itself part of an *SphI* site which is suitable for cloning heterologous genes at their initiating methionine. Downstream of the *SphI* site, the *EcoRI, NotI,* and *XhoI* sites are retained.

An alternative vector is constructed which utilizes an *NcoI* site at the initiating ATG. This vector, designated pCGN1761NENX is made by inserting an annealed molecular adaptor of the sequence 5'-AATTCTAAACCATGGCGATCGG-3' (SEQ ID NO:5) / 5'AATTCCGATCGCCATGGTTTA-3' (SEQ ID NO:6) at the pCGN1761ENX *EcoRI* site. Thus, the vector includes the *quasi*-

optimized sequence TAAACC adjacent to the initiating ATG which is within the *NcoI* site. Downstream sites are *EcoRI, NotI,* and *XhoI.* Prior to this manipulation, however, the two *NcoI* sites in the pCGN1761ENX vector (at upstream positions of the 5' 35S promoter unit) are destroyed using similar techniques to those described above for *SphI* or alternatively using "inside-outside" PCR (Innes *et al.* PCR Protocols: A guide to methods and applications. Academic Press, New York (1990). This manipulation can be assayed for any possible detrimental effect on expression by insertion of any plant cDNA or reporter gene sequence into the cloning site followed by routine expression analysis in plants.

On page 32, please amend paragraph 2 to read as follows:

A preferred vector is constructed by transferring the *DraI-SphI* transit peptide encoding fragment from prbcS-8B to the cloning vector pCGN1761ENX/Sph-. This plasmid is cleaved with *EcoRI* and the termini rendered blunt by treatment with T4 DNA polymerase. Plasmid prbcS-8B is cleaved with *SphI* and ligated to an annealed molecular adaptor of the sequence 5'-CCAGCTGGAATTCCG-3' (SEQ ID NO:7) / 5'-CGGAATTCCAGCTGGCATG-3' (SEQ ID NO:8). The resultant product is 5'-terminally phosphorylated by treatment with T4 kinase. Subsequent cleavage with *DraI* releases the transit peptide encoding fragment which is ligated into the blunt-end ex-*EcoRI* sites of the modified vector described above. Clones oriented with the 5' end of the insert adjacent to the 3' end of the 35S promoter are identified by sequencing. These clones carry a DNA fusion of the 35S leader sequence to the *rbcS-8A* promoter-transit peptide sequence extending from -58 relative to the *rbcS* ATG to the ATG of the mature protein, and including at that position a unique *SphI* site, and a newly created *EcoRI* site, as well as the existing *NotI*

and *XhoI* sites of pCGN1761ENX. This new vector is designated pCGN1761/CT. DNA sequences are transferred to pCGN1761/CT in frame by amplification using PCR techniques and incorporation of an *SphI, NSphI,* or *NlaIII* site at the amplified ATG, which following restriction enzyme cleavage with the appropriate enzyme is ligated into *SphI*-cleaved pCGN1761/CT. To facilitate construction, it may be required to change the second amino acid of cloned gene, however, in almost all cases the use of PCR together with standard site directed mutagenesis will enable the construction of any desired sequence around the cleavage site and first methionine of the mature protein.

On page 39, please amend paragraph 2 to read as follows:

Example A1: Preparation of a chimeric gene containing the T. fusca E1 cellulase coding sequence fused to the tobacco PR-1a promoter

Plasmid pGFE1 (Jung et al. (1993) Appl. Environ. Microbiol. 59, 3032-3043) containing the T. fusca E1 gene (GenBank accession number L20094), which codes for a protein with endoglucanase activity, was used as the template for PCR with a left-to-right "top strand" primer comprising an ATG before the first codon of the mature E1 protein, the first 21 base pairs of the mature protein and a *NcoI* restriction site at the newly created ATG (primer E11: GCG CCC ATG GAC GAA GTC AAC CAG ATT CGC) (SEQ ID NO:9) and a right-to-left "bottom strand" primer homologous to positions 322 to 346 from the newly created ATG of the E1 gene (primer E12: CCA GTC GAC GTT GGA GGT GAA GAC) (SEQ ID NO:10). This PCR reaction was undertaken with AmpliTaq DNA polymerase according to the manufacturer's recommendations (Perkin Elmer/Roche, Branchburg, NJ) for five cycles at 94°C (30 s), 40°C (60 s), and 72°C (30 s) followed by 25 cycles at 94°C (30 s), 55°C (60 s) and 72°C (30 s). This generated a product of 352 bp containing a *NcoI* site at its left

end and a *EcoRI* site at its right end and comprised the 5' end of the E1 gene without the signal sequence. The fragment was gel purified using standard procedures, cleaved with *NcoI* and *EcoRI* (all restriction enzymes purchased from Promega, Madison, WI or New England Biolabs, Beverly, MA) and ligated into the *NcoI* and *EcoRI* sites of pTC191 (De La Fuente *et al.* (1994) Gene 139, 83-86) to obtain pE1.

Please amend the paragraph bridging pages 40 and 41 to read as follows:

Example A2: Preparation of a chimeric gene containing the T. fusca E2 cellulase coding sequence fused to the tobacco PR-1a promoter

Plasmid pJT17 containing the T. fusca E2 gene (Ghangas et al. (1988) Appl. Environ. Microbiol. 54, 2521-2526; Lao et al. (1991) J. Bacteriol. 173, 3397-3407) (GenBank accession number M73321), which codes for a protein with cellobiohydrolase activity, was used as the template for PCR with a left-to-right "top strand" primer comprising an ATG before the last codon of the E2 signal sequence, the first 18 base pairs of the mature protein and a *NcoI* restriction site at the newly created ATG (primer E21: GCG CGC CAT GGC CAA TGA TTC TCC GTT CTA C) (SEQ ID NO:11) right-to-left "bottom strand" primer homologous to positions 310 to 334 from the newly created ATG of the E2 gene (primer E22: GGG ACG GTT CTT CAG TCC GGC AGC) (SEQ ID NO:12). This PCR reaction was undertaken with AmpliTaq DNA polymerase according to the manufacturer's recommendations for five cycles at 94°C (30 s), 40°C (60 s), and 72°C (30 s) followed by 25 cycles at 94°C (30 s), 55°C (60 s) and 72°C (30 s). This generated a product of 341 bp containing a *NcoI* site at its left end and a *EcoRI* site at its right end comprising the 5' end of the E2 gene without a signal sequence. The fragment was gel purified using standard procedures, cleaved with *NcoI* and *EcoRI* and ligated into the *NcoI* and *EcoRI* sites of pTC191 to obtain pE2.

Please amend the paragraph bridging pages 41 and 42 to read as follows:

Example A3: Preparation of a chimeric gene containing the T. fusca E5 cellulase coding sequence fused to the tobacco PR-1a promoter

Plasmid pD374, a modified version of pD370 (Collmer and Wilson (1983) Biotechnology 1, 594-601; Lao et al. (1991) J. Bacteriol. 173, 3397-3407) containing the T. fusca E5 gene (GenBank accession number L01577), which codes for a protein with endoglucanase activity, was used as the template for PCR with a left-to-right "top strand" primer comprising an ATG before the first codon of the mature E5 protein, the first 21 base pairs of the mature protein and a *NcoI* restriction site at the newly created ATG (primer E51: CGC CCA TGG CCG GTC TCA CCG CCA CAG TC) (SEQ ID NO:13) and a right-to-left "bottom strand" primer homologous to positions 89 to 114 from the newly created ATG of the E5 gene (primer E52: GAC GAC CTC CCA CTG GGA GAC GGT G) (SEQ ID NO:14). AmpliTaq DNA polymerase was used for PCR according to the manufacturer's recommendations for five cycles at 94°C (30 s), 40°C (60 s), and 72°C (30 s) followed by 25 cycles at 94°C (30 s), 55°C (60 s) and 72°C (30 s). This generated a product of 119 bp containing a *NcoI* site at its left end and a *XhoI* site at its right end and comprised the 5' end of the E5 gene without a signal sequence. The fragment was gel purified, cleaved with *NcoI* and *XhoI* and ligated into the *NcoI* and *XhoI* sites of pCIB4247 to obtain pCE5. pCIB4247 is a pUC19 derivative (Yanisch-Perron *et al.* (1985) Gene 33, 103-119) containing a polylinker with *NcoI*, *XhoI* and *EcoRI* restriction sites.

Please amend the paragraph bridging pages 46 and 47 to read as follows:

Example B1: Preparation of a chimeric gene containing the T. fusca E5 cellulase coding sequence fused to the tobacco PR-1a promoter

Plasmid pD374 containing the T. fusca E5 gene (see Example A3) was used as template for PCR with a left-to-right "top strand" primer extending from position 1,135 to 1,156 in the E5 gene relative to the endogenous ATG and comprising an additional *NcoI* site at its left end (primer VAC1: CAT GCC ATG GGT GAG GCC TCC GAG CTG TTC C) (SEQ ID NO:15) and a right-to-left "bottom strand" primer whose sequence was homologous to the 21 last bp of the E5 gene and including 21 bp of a vacuolar targeting sequence derived from a tobacco chitinase gene (Shinshi et al. (1990) Plant Mol. Biol. 14, 357-368, Neuhaus et al. (1991) Proc. Natl. Acad. Sci. USA 88, 10362-10366), the stop codon of the same tobacco chitinase gene and a *SacI* restriction site (primer VAC2: TGC GAG CTC TTA CAT AGT ATC GAC TAA AAG TCC GGA CTG GAG CTT GCT CCG CAC) (SEQ ID NO:16). AmpliTaq DNA polymerase was used for PCR according to the manufacturer's recommendations for five cycles at 94°C (30 s), 40°C (60 s), and 72°C (30 s) followed by 25 cycles at 94°C (30 s), 55°C (60 s) and 72°C (30 s). This generated a product of 283-bp containing the 3' end of the E5 gene fused to the vacuolar targeting sequence. The fragment was gel purified, cleaved with *NcoI* and *SacI* and ligated into the *NcoI* and *SacI* sites of pJG203 to obtain pJGDE5.

Please amend the paragraph bridging pages 52 and 53 to read as follows:

Example C2: Preparation of a modified tobacco plastid transformation vector containing the T. fusca E5 cellulase coding sequence fused to a modified bacteriophage T7 gene 10 promoter and terminator engineered for reduced read-through transcription

Plasmid pC8 was digested with *SpeI* and *NcoI* and a 235 bp fragment containing the T7 gene 10 promoter and a portion of the

divergent psbA gene promoter and 5' UTR was isolated by gel purification and cloned into the *NcoI* and *SpeI* restriction sites of vector pGEM5Zf+ (Promega, Madison WI) to construct plasmid pPH118. pPH118 was digested with *StuI* and the 3210 bp vector fragment gel purified and religated to construct plasmid pPH119 which lacks the duplicated 10 bp sequence CGAGGCCTCG (SEQ ID NO:17) (*StuI* site underlined) that was found by sequence analysis to be present in plasmid pC8. Elimination of the 10 bp *StuI/StuI* fragment in pPH119 was verified by sequencing using universal M13 forward and reverse primers.

Please amend the paragraph bridging pages 53 and 54 to read as follows:

Example C3: Construction of a plastid-targeted bacteriophage T7 RNA polymerase gene fused to the tobacco PR-1a promoter

A synthetic oligonucleotide linker comprising an *NcoI* restriction site and ATG start codon followed by the first seven plastid transit peptide codons from the rbcS gene (encoding the small subunit of ribulose bisphosphate carboxylase) and endogenous *PstI* restriction site (top strand: 5'-CAT GGC TTC CTC AGT TCT TTC CTC TGC A-3' (SEQ ID NO:18); bottom strand: 5'-GAG GAA AGA ACT GAG GAA GC-3' (SEQ ID NO:19)), a 2.8 kb *PstI/SacI* DNA fragment of pCGN4205 (McBride, K. E. et al. (1994) PNAS 91, 7301-7305) containing the bacteriophage T7 RNA polymerase gene (T7 Pol) fused in frame to the 3' portion of the rbcS gene transit peptide coding sequence, a 0.9 kb *NcoI/KpnI* DNA fragment of pCIB296 containing the tobacco PR-1a promoter with an introduced *NcoI* restriction site at the start codon (Uknes et al. (1993) Plant Cell 5, 159 169) and 4.9 kb *SfiI/KpnI* and 6.6 kb *SacI*/SfiI fragments of binary Agrobacterium transformation vector pSGCGC1 (a derivative of pGPTV-Hyg